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Frequent PD-L1 expression in testicular germ cell tumors

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Abstract: BACKGROUND Many testicular germ cell cancers are curable despite metastatic disease, but about 10-15% of patients fail cisplatin-based first-line treatment. Immunotherapy is considered as additional treatment approach for these patients. Inhibition of the interaction between Programmed Death Receptor 1 (PD-1) and Programmed Death Receptor Ligand 1 (PD-L1) enhances T-cell responses in vitro and mediates clinical antitumour activity. We analysed the expression of PD-L1 in testicular germ cell tumours to evaluate its potential as target for immunotherapeutic strategies. METHODS Immunohistochemistry was performed in 479 formalin-fixed paraffin-embedded specimens using a rabbit monoclonal antibody (E1L3N). The tissue microarray consisted of 208 pure seminomas, 121 non-seminomas, 20 intratubular germ cell neoplasia unclassified (IGCNU) and 20 specimens of non-neoplastic testicular tissue. RESULTS Programmed Death Receptor Ligand-1 expression was found in 73% of all seminomas and in 64% of all non-seminomas. None of 20 IGCNU and none of 20 normal tissue specimens exhibited PD-L1 expression. PD-L1 positive stromal cells were only detected in seminomas, but not in non-seminomas. The anti PD-L1 antibody showed a pre-dominantly membranous staining pattern in testicular tumour cells, as well as expression in stromal cells. CONCLUSIONS This frequent expression of PD-L1 in human testicular germ cell tumours suggests that patients with testicular germ cell tumours could profit from immunotherapeutic strategies using anti-PD1 and anti-PDL1 antibodies.

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Frequent PD-L1 expression in testicular germ cell tumors

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Background: Many testicular germ cell cancers are curable despite metastatic disease, but about 10–15% of patients fail cisplatin-based first-line treatment. Immunotherapy is considered as additional treatment approach for these patients. Inhibition of the interaction between Programmed Death Receptor 1 (PD-1) and Programmed Death Receptor Ligand 1 (PD-L1) enhances T-cell responses *in vitro* and mediates clinical antitumour activity. We analysed the expression of PD-L1 in testicular germ cell tumours to evaluate its potential as target for immunotherapeutic strategies.

Methods: Immunohistochemistry was performed in 479 formalin-fixed paraffin-embedded specimens using a rabbit monoclonal antibody (E1L3N). The tissue microarray consisted of 208 pure seminomas, 121 non-seminomas, 20 intratubular germ cell neoplasia unclassified (IGCNU) and 20 specimens of non-neoplastic testicular tissue.

Results: Programmed Death Receptor Ligand-1 expression was found in 73% of all seminomas and in 64% of all non-seminomas. None of 20 IGCNU and none of 20 normal tissue specimens exhibited PD-L1 expression. PD-L1 positive stromal cells were only detected in seminomas, but not in non-seminomas. The anti PD-L1 antibody showed a pre-dominantly membranous staining pattern in testicular tumour cells, as well as expression in stromal cells.

Conclusions: This frequent expression of PD-L1 in human testicular germ cell tumours suggests that patients with testicular germ cell tumours could profit from immunotherapeutic strategies using anti-PD1 and anti-PDL1 antibodies.

Testicular germ cell tumours are curable despite the presence of metastatic disease. Nevertheless, about 10–15% of patients fail cisplatin-based first-line chemotherapy and about 3–5% of all patients with testicular germ cell tumours will eventually die of their disease. Moreover, short- and long-term side effects of cisplatin-based chemotherapy make the search for new and less toxic treatment strategies worthwhile.

Programmed Death Receptor 1 (PD-1, CD279) is one of the members of the extended family of T-cell regulators expressed on the surface of activated T cells, B cells and macrophages (Keir *et al*, 2008). Its ligand, Programmed Death Receptor Ligand 1 (PD-L1) (B7-H1, CD274), is expressed on tumour cells, macrophages, T cells and certain other tissue types (Keir *et al*, 2008). The interaction of these two molecules negatively regulates immune responses. Of major interest is that inhibition of the interaction between PD1 and PD-L1 can enhance T-cell

responses *in vitro* and mediates clinical antitumour activity (Berger *et al*, 2008). PD-L1 expression in tumour specimens has been described as a predictive marker for tumour response to anti-PD1 or -PD-L1 immunotherapy in various advanced tumours, including melanoma, non-small cell lung cancer, kidney cancer, colorectal cancer, castration-resistant prostate cancer and bladder cancer (Berger *et al*, 2008). For example, in bladder cancer, a disease that has not seen therapeutic advances for several decades, the anti-PD-L1 antibody MPDL3280A demonstrate antitumour responses with objective response rates up to 53% in patients with PD-L1-positive tumours and 13% in PD-L1-negative tumours (Powles *et al*, 2014). In metastatic melanoma one-third showed objective tumour regressions to the anti PD-1 agent Nivolumab with a median response duration of 2 years (Topalian *et al*, 2014). The aim of this study was to investigate the expression of PD-L1 in testicular germ cell tumours.

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MATERIALS AND METHODS

Formalin-fixed paraffin-embedded tumour specimens from 329 patients diagnosed with primary testicular germ cell tumours were retrieved from the Institute of Surgical Pathology of the University Hospital Zurich, Switzerland from 1990 to 2003. The patient age ranged from 18 to 90 with a median of 33.5 years. Tumours were classified according to the 2004 WHO Classification. A tissue microarray was constructed and included a total of 208 pure seminomas and 121 non-seminomas or mixed tumours as described previously (Bode *et al*, 2011). In mixed germ cell tumours, each tumour component (seminomatous, embryonal carcinoma, yolk sac tumour, choriocarcinoma, teratoma) was separately punched. Briefly, the tissue microarray consisted of the following tumour components: 248 seminomas, 87 embryonal carcinomas, 48 yolk sac tumours, 46 teratomas and 10 choriocarcinomas. Furthermore, 20 samples of normal testicular tissue as well as 20 samples of intratubular germ cell neoplasia unclassified were included.

To detect the PD-L1 protein, we used the monoclonal rabbit antibody (E1L3N, Cell Signaling Technology, Inc. (CST), Danvers, MA, USA). A multi-tumour tissue microarray was used to establish a staining protocol for the PD-L1 antibody. A dilution of 1:1000 resulted in a strong and distinct membranous signal without unspecific background staining in positive controls (PD-L1-positive lung cancer cases). Programmed Death Receptor Ligand-1-negative lung cancer cases were used as negative controls. An experienced uropathologist (PKB) evaluated all tissue microarray spots. All results were re-evaluated by a second observer (CDF). In discrepant cases, consensus was achieved between the two observers after individual case discussion. Percentages of PD-L1-positive tumour cells and staining pattern were evaluated for each punch. Programmed Death Receptor Ligand-1 expression was recorded if a distinct membranous staining signal on the tumour cell surface or strong cytoplasmic staining within the tumour or

stromal cells was observed. A 5% cut-off value was applied for PD-L1 positivity as it has been proposed in non-small cell lung cancer (Zhang *et al*, 2015). To evaluate the overall tumour expression of non-seminomas, tumours with multiple components were considered PD-L1-positive if any component met these criteria.

RESULTS

Programmed Death Receptor Ligand-1 expression was found in 73% of seminomas and 64% of non-seminomas. The expression in the individual tumour components is shown in Figure 1 and summarised in Table 1. None of the 20 precursor lesions and none of the 20 normal testicular specimens exhibited PD-L1 expression. Correlation with tumour stage showed PD-L1 expression in 53% (pT1), 66% (pT2) and 70% (pT3).

In general, PD-L1 expression was found to be heterogeneous in the tumour tissue showing dispersed positive foci. Furthermore, differences between seminoma and non-seminoma were noted. The seminoma components showed a positive staining predominantly in the interface region between tumour and fibrovascular stromal septae. Frequently, some seminoma cells showed a distinct membranous staining. In the stromal septae, it was difficult to evaluate which exact cells (lymphocytes, stromal cells, endothelial cells) were positive because of the marked density of the cellular infiltrate in the stroma, but it seemed that it was mainly restricted to the lymphocytes. Most cases of non-seminoma components showed a distinct strong membranous staining of the tumour cells and only rarely expression in the tumour stroma was noted.

DISCUSSION

In this study, we observed frequent PD-L1 expression in testicular germ cell tumours, but not in their precursor lesion (intratubular

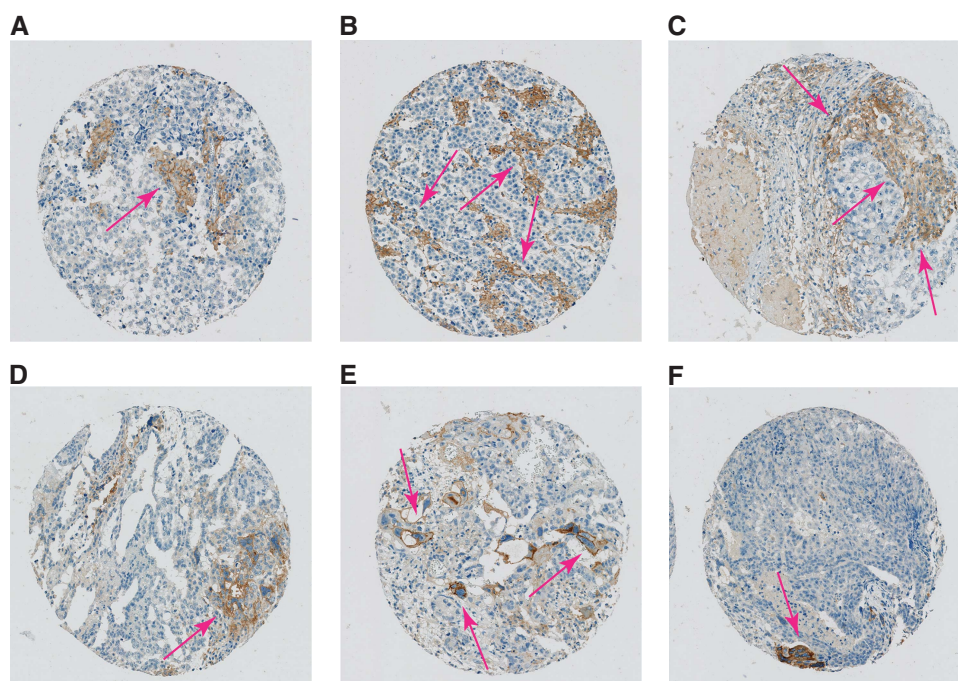


Figure 1. Programmed cell death ligand 1 (PD-L1) staining in several tumour components: Classic seminoma with distinct membrane staining in a small focus (A); Classic seminoma with typical stromal staining pattern (B); Embryonal carcinoma with typical membranous staining pattern (C); Yolk sac tumour with distinct membrane staining in a small focus (D); choriocarcinoma with strong membrane staining in dispersed tumour cells, predominantly multinucleated giant cells (E); Embryonal carcinoma with a small focus demonstrating the marked heterogeneity of PD-L1 expression (F).

Table 1. Summary of PD-L1 expression for individual tissue types, tumour components and tumour stage

| Tissue types | Negative | Weak | Strong | Positive cases (%) |
|-------------------------------------|----------|------|--------|--------------------|
| Seminoma (n = 208) | 57 | 67 | 84 | 73% |
| Non-seminoma (n = 121) | 43 | 35 | 43 | 64% |
| Intratubular germ cell neoplasia | 20 | 0 | 0 | 0% |
| Normal testis | 20 | 0 | 0 | 0% |
| Individual tumour components | | | | |
| Seminomatous (n = 248) | 77 | 77 | 94 | 69% |
| Choriocarcinoma (n = 10) | 2 | 2 | 6 | 80% |
| Yolk sac tumour (n = 48) | 29 | 12 | 7 | 40% |
| Embryonal carcinoma (n = 87) | 34 | 29 | 24 | 61% |
| Teratoma (n = 46) | 39 | 5 | 2 | 13% |
| Tumour stage | | | | |
| pT1 (n = 352) | 164 | 83 | 105 | 53% |
| pT2 (n = 94) | 32 | 38 | 24 | 66% |
| pT3 (n = 10) | 3 | 3 | 4 | 70% |

germ cell neoplasia unclassified). Importantly, normal testis was PD-L1 negative, too. The frequent expression of PD-L1 in this cohort is comparable with other genitourinary cancer like renal cell carcinoma (66%) (Thompson *et al*, 2005) and bladder cancer (20%) (Faraj *et al*, 2015) or non-genitourinary cancers like breast cancer (23.4%) (Muenst *et al*, 2014) or colorectal cancer (36%) (Droeser *et al*, 2013). In these tumours, the positive expression of PD-L1 has been described to be associated with shorter overall survival (Zhang *et al*, 2015). Furthermore, clinical trials suggested that the tissue expression status of PD-L1 is the most important marker to predict therapy response to PD-1 and PD-L1 immunotherapy (Berger *et al*, 2008). A major limitation of our study might be the lack of clinical follow-up data. Nevertheless, our accurate description of PD-L1 expression in testicular germ cell tumours forms the basis for a potential use of PD-L1 as predictive marker in future PD-L1 immunotherapies.

We conclude that the frequent expression of PD-L1 in testicular seminomas and non-seminomas indicates that immune checkpoint blockade could be a promising new treatment approach also in testicular cancer that warrants further investigation in clinical trials.

Take home message. Frequent PD-L1 expression in testicular germ cell tumours indicates that PD-L1 immunotherapy might be a novel option in the treatment of testicular cancer.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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